

WHAT IS CLAIMED IS:

- 1                   1.       A bifunctional polypeptide comprising a binding ligand linked to a first  
2 member of a coil-coil binding pair and a reporter molecule linked to the second member of a  
3 coil-coil binding pair, wherein binding between the first coil domain and the second coil  
4 domain joins the binding ligand to the reporter molecule.
- 1                   2.       The bifunctional polypeptide of claim 1, wherein the coil-coil binding  
2 pair is an E coil and a K coil.
- 1                   3.       The bifunctional polypeptide of claim 1, wherein the coil-coil binding  
2 pair is an A coil and a B coil.
- 1                   4.       The bifunctional polypeptide of claim 1, wherein the first and the  
2 second members of the coil-coil binding pair are each at least 35 amino acids in length.
- 1                   5.       The bifunctional polypeptide of claim 1, wherein the binding ligand is  
2 an antibody selected from the group consisting of an scFv, an Fab fragment, an isolated V<sub>H</sub>,  
3 and an isolate V<sub>L</sub>.
- 1                   6.       The bifunctional polypeptide of claim 1, wherein the binding ligand is  
2 a peptide.
- 1                   7.       The bifunctional polypeptide of claim 1, wherein the binding ligand is  
2 a receptor.
- 1                   8.       The bifunctional polypeptide of claim 1, wherein the reporter molecule  
2 is a fluorescent protein or chromophoric protein.
- 1                   9.       The bifunctional polypeptide of claim 8, wherein the fluorescent  
2 protein is green fluorescent protein.
- 1                   10.      The bifunctional polypeptide of claim 8, wherein the fluorescent  
2 protein is red fluorescent protein.
- 1                   11.      The bifunctional polypeptide of claim 1, wherein the reporter molecule  
2 is a fluorescent dye.

1                   12.     The bifunctional polypeptide of claim 1, wherein the reporter molecule  
2     is an enzyme.

1                   13.     The bifunctional polypeptide of claim 12, wherein the enzyme is  
2     horseradish peroxidase.

1                   14.     The bifunctional polypeptide of claim 12, wherein the enzyme is  
2     alkaline phosphatase.

1                   15.     The bifunctional polypeptide of claim 1, wherein the reporter molecule  
2     is a biotin binding protein.

1                   16.     The bifunctional polypeptide of claim 1, wherein the reporter molecule  
2     has luminescent activity.

1                   17.     The bifunctional polypeptide of claim 16, wherein the reporter  
2     molecule is luciferase.

1                   18.     A multifunctional polypeptide comprising:  
2                   a first member of a coil-coil binding pair linked to a binding ligand; and  
3                   a second member of the coil-coil binding pair linked to a polypeptide that  
4     undergoes spontaneous multimerization to form a self-assembled complex;  
5                   wherein binding between the first member of the coil-coil binding pair and the  
6     second member of the coil-coil pair joins the binding ligand and the self-assembled complex.

1                   19.     The multifunctional polypeptide of claim 18, further comprising a  
2     reporter molecule that is individually linked to a first member of the coil-coil binding pair,  
3     wherein the coil-coil binding interaction joins the reporter molecule to the multifunctional  
4     polypeptide.

1                   20.     The multifunctional polypeptide of claim 18, further comprising a  
2     second binding ligand that is individually linked to a first member of a coil-coil binding pair,  
3     wherein the second binding ligand binds to an epitope different from the first binding ligand,  
4     and wherein the coil-coil binding interaction joins the self-assembled complex to the first and  
5     second binding ligands.

- 1                    21.    The multifunctional polypeptide of claim 18, further comprising a  
2 second polypeptide individually linked to a second member of the coil-coil binding pair,  
3 wherein the second polypeptide undergoes spontaneous multimerization with the first  
4 polypeptide to form the self-assembled complex, and wherein the coil-coil binding interaction  
5 joins the self-assembled complex to the binding ligand.
- 1                    22.    The multifunctional polypeptide of claim 18, wherein the polypeptide  
2 is a soluble ferritin subunit.
- 1                    23.    The multifunctional polypeptide of claim 18, wherein the polypeptide  
2 is a viral coat protein.
- 1                    24.    The multifunctional polypeptide of claim 18, wherein the coil-coil  
2 binding pair is an E coil and a K coil.
- 1                    25.    The multifunctional polypeptide of claim 18, wherein the coil-coil  
2 binding pair is an A coil and a B coil.
- 1                    26.    The multifunctional polypeptide of claim 18, wherein the first and the  
2 second members of the coil-coil binding pair are each at least 35 amino acids in length.
- 1                    27.    The multifunctional polypeptide of claim 18, wherein the binding  
2 ligand is an antibody selected from the group consisting of a single chain Fv, an Fab, an  
3 isolated V<sub>H</sub>, and an isolated V<sub>L</sub>.
- 1                    28.    The multifunctional polypeptide of claim 18, wherein the binding  
2 ligand is a peptide.
- 1                    29.    The multifunctional polypeptide of claim 18, wherein the binding  
2 ligand is a fluorobody.
- 1                    30.    The multifunctional polypeptide of claim 18, wherein the binding  
2 ligand is a receptor.
- 1                    31.    The multifunctional polypeptide of claim 18, wherein the reporter  
2 molecule is a fluorescent protein.

1                   32.     The multifunctional polypeptide of claim 18, wherein the fluorescent  
2 protein is green fluorescent protein.

1                   33.     The multifunctional polypeptide of claim 18, wherein the fluorescent  
2 protein is red fluorescent protein.

1                   34.     The multifunctional polypeptide of claim 18, wherein the reporter  
2 molecule is an enzyme.

1                   35.     The multifunctional polypeptide of claim 18, wherein the reporter  
2 molecule is a biotin binding protein.

1                   36.     The multifunctional polypeptide of claim 18, wherein the reporter  
2 molecule is luciferase.

1                   37.     A kit comprising:  
2 a binding ligand linked to a first member of a coil-coil binding pair; and  
3 a reporter molecule linked to a second member of a coil-coil binding pair.

1                   38.     The kit of claim 37, wherein the binding ligand is an antibody.

1                   39.     The kit of claim 37, wherein the binding ligand is a peptide.

1                   40.     The kit of claim 37, wherein the binding ligand is a fluorobody.

1                   41.     The kit of claim 37, wherein the reporter molecule is a fluorescent  
2 protein.

1                   42.     The kit of claim 37, wherein the reporter molecule is an enzyme.

1                   43.     The kit of claim 37, wherein the reporter molecule is a biotin binding  
2 protein.

1                   44.     The kit of claim 37, wherein the reporter molecule is luciferase.

1                   45.     A kit comprising:  
2 a first subunit that is a first member of a coil-coil binding pair linked to a  
3 binding ligand; and

4                   a second subunit that is a second member of the coil-coil binding pair linked to  
5 a polypeptide that undergoes spontaneous multimerization.

1                   46.     The kit of claim 45, further comprising a third subunit that is a first  
2 member of the coil-coil binding pair linked to a reporter molecule.

1                   47.     The kit of claim 45, wherein the polypeptide that undergoes  
2 spontaneous multimerization is soluble ferritin.

1                   48.     The kit of claim 45, wherein the polypeptide that undergoes  
2 spontaneous multimerization is a viral coat protein.

1                   49.     The kit of claim 45, wherein the binding ligand is an antibody selected  
2 from the group consisting of an scFV, an Fab, a V<sub>H</sub> region and a V<sub>L</sub> region.

1                   50.     The kit of claim 45, wherein the binding ligand is a fluorobody.

1                   51.     The kit of claim 45, wherein the binding ligand is a peptide.

1                   52.     The kit of claim 45, wherein the binding ligand is a receptor.

1                   53.     The kit of claim 45, wherein the reporter molecule is a polypeptide  
2 selected from the group consisting of a fluorescent protein and an enzyme.

1                   54.     The kit of claim 53, wherein the reporter molecule is a fluorescent  
2 protein or an enzyme.

1                   55.     A method of making a multifunctional polypeptide, the method  
2 comprising:  
3                   providing a binding ligand linked to a first member of a coil-coil binding pair;  
4                   providing a molecule linked to a second member of a coil-coil binding pair,  
5 wherein the molecule is a reporter molecule or a spontaneously multimerizing polypeptide;  
6 and  
7                   incubating the binding pair under conditions in which the first binding pair  
8 member specifically binds to the second binding pair member, thereby assembling the  
9 bifunctional polypeptide.

1                    56.     A method of screening for the presence of an antigen, the method  
2 comprising:  
3                    incubating a sample comprising the antigen with a bifunctional polypeptide  
4 comprising a binding ligand linked to a first member of a coil-coil binding pair and a reporter  
5 molecule linked to the second member of the coil-coil binding pair, wherein the binding  
6 ligand is joined to the reporter polypeptide by the binding interaction between the binding  
7 pair members;  
8                    wherein the antigen and the bifunctional polypeptide are incubated under  
9 conditions in which the antigen specifically binds to the binding ligand; and  
10                    detecting activity of the reporter molecule, thereby detecting the presence of  
11 the antigen.

1                    57.     A method of screening for the presence of an antigen, the method  
2 comprising:  
3                    incubating a sample comprising the antigen with a binding ligand linked to a  
4 first member of a coil-coil binding pair under conditions in which the antigen specifically  
5 binds to the binding ligand and subsequently incubating the sample with a reporter molecule  
6 linked to the second member of the coil-coil binding pair, wherein the binding ligand  
7 becomes joined to the reporter molecule by the binding interaction between the binding pair  
8 members; and  
9                    detecting activity of the reporter polypeptide, thereby detecting the presence of  
10 the antigen.

1                    58.     A method of screening for the presence of an antigen, the method  
2 comprising:  
3                    (a) incubating a sample comprising the antigen with a bifunctional polypeptide  
4 comprising:  
5                    (i) a binding ligand linked to a first member of a coil-coil binding pair;  
6 and  
7                    (ii) a polypeptide that undergoes spontaneous multimerization linked to  
8 the second member of a coil-coil binding pair, wherein binding between the first coil domain  
9 and the second coil domain joins the binding ligand and the spontaneously multimerizing  
10 polypeptide,

11                                wherein the antigen and the bifunctional polypeptide are incubated  
12 under conditions in which the antigen specifically binds to the binding ligand; and  
13                                (b) detecting the presence of the bifunctional polypeptide, thereby detecting  
14 the presence of the antigen.

1                                59.     A method of screening for the presence of an antigen, the method  
2 comprising:  
3                                incubating a sample comprising the antigen with a binding ligand linked to a  
4 first member of a coil-coil binding pair under conditions in which the antigen specifically  
5 binds to the binding ligand and subsequently incubating the sample with a second  
6 polypeptide linked to a second member of the coil-coil binding pair, wherein the second  
7 polypeptide undergoes spontaneous multimerization;  
8                                wherein the binding ligand is joined to the second polypeptide by the  
9 binding interaction between the coil-coil binding pair members to form a multifunctional  
10 polypeptide; and  
11                                detecting the presence of the multifunctional polypeptide, thereby detecting  
12 the presence of the antigen.